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# Solid-Phase Radioimmunossay for Rapid Detection and Identification of Western Equine Encephalomyelitis Virus

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A solid-phase radioimmunoassay technique was adapted for the rapid detection and identification of western equine encephalomyelitis virus in clinical specimens.

Radioimmunoassay (RIA) procedures for detection of viral antigens and antibodies have been found to be more sensitive than conventional serological techniques (2, 3, 6, 9). In addition, it has been shown that $^{125}$I-labeled antibodies can be used to detect herpesvirus antigens on the surface of infected cells (7, 8). Applying this finding, Forghani et al. (2) developed a solid-phase RIA procedure for the type-specific identification of herpesvirus types 1 and 2 in clinical specimens. We have recently adapted this procedure for the rapid identification of western equine encephalomyelitis (WEE) virus.

A schematic diagram illustrating the procedure developed in this study is shown in Fig. 1. Glass vials (1 by 3 cm; A. H. Thomas, Co., Philadelphia, Pa.) containing confluent monolayers of primary duck embryo cells (DEC) were inoculated with 0.1 ml of DEC-grown WEE virus (strain 628). After a 2-h adsorption period, the specimens were removed and 1.0 ml of medium 199 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 2% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 $\mu$g/ml) was added to each vial. The vials were then incubated overnight at 37°C in a 5% $CO_2$ atmosphere. After incubation, the medium was removed and the monolayers were rinsed with a balanced salt solution and fixed with cold acetone for 10 min to expose any intracellular viral antigen present. Once the vials were dry, 0.05 ml of rabbit hyperimmune WEE antiserum was added; vials were incubated at 37°C for 1 h. The optimal antiserum concentration used in these experiments was determined previously by block titration. The serum was then removed and the vials were rinsed three times to remove residual, unabsorbed antibody. Goat anti-rabbit gamma globulin was obtained from Cappel Laboratories, Downington, Pa. The antiglobulin was labeled by the lactoperoxidase technique of Marchalonis (5) after the addition of $^{125}$I (New England Nuclear Corp., Boston, Mass.) in carrier-free form at a concentration of 400 $\mu$Ci per mg of protein. A 0.1-ml volume of labeled goat anti-rabbit gamma globulin that contained approximately 100,000 cpm, a concentration previously shown to be optimal for detection of cell-bound rabbit globulin, was added to each vial. After incubation at 37°C for 1 to 2 h, the vials were rinsed five times with balanced salt solution and placed in counting tubes; residual radioactivity was assayed with a gamma counter (Nuclear-Chicago, Des Plaines, Ill.). All experiments included controls that consisted of uninfected cells and preimmunization sera. Binding ratios were expressed as the mean counts per minute in three infected cultures divided by the

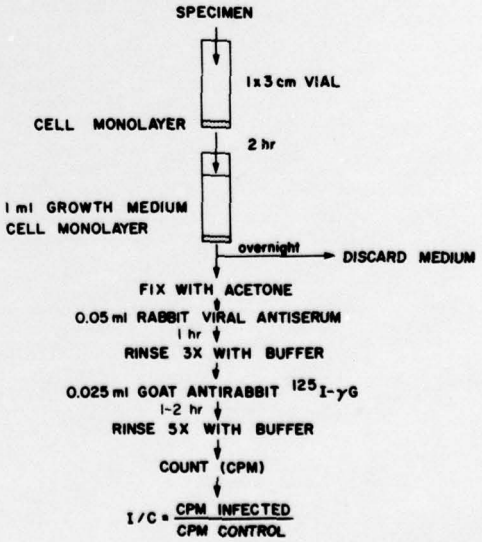


FIG. 1. *Flow sheet illustrating the solid-phase RIA procedure for detection and identification of WEE virus.*

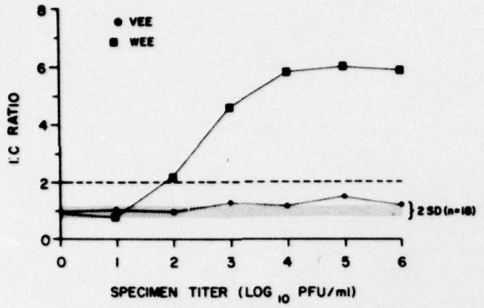


FIG. 2. *Binding ratios obtained in a solid-phase RIA with a homologous WEE virus and heterologous VEE virus. The stippled band represents the standard error calculated about the mean (5,300 cpm) of 18 uninfected control vials and represents the variation among replicate samples. SD, standard deviation; PFU, plaque-forming units.*

mean counts per minute in corresponding uninfected cultures. Elapsed time from specimen inoculation to final counting was less than 24 h.

The results from a typical experiment demonstrate the sensitivity and specificity of this identification procedure (Fig. 2). Replicate cell cultures were infected with virus concentrations ranging from 1 to 6 logs of either WEE or Venezuelan equine encephalomyelitis (VEE) virus. After overnight incubation, the cultures were tested by RIA as described above. All binding ratios equal to or greater than 2 were considered positive. It can be seen that when rabbit anti-WEE serum was used, cultures inoculated with WEE virus (>2 logs/ml) were recorded as positive. In contrast, the heterologous virus, VEE, shown previously to propagate in DEC cultures (4), resulted in binding ratios less than 2 at all concentrations used.

A final experiment was performed to examine the feasibility of using this solid-phase RIA for the detection and identification of WEE virus in clinical specimens. Tissue specimens from WEE virus (clone 15 strain)-infected and noninfected fetuses of rhesus monkeys were examined in parallel for virus content by both plaque assay and RIA. Tissue homogenizers were used to prepare a 20% suspension of each tissue. These suspensions were centrifuged and used as inocula for virus isolation by plaque assay on DEC monolayers. These same suspensions were examined for virus content by RIA. All specimens from infected tissues demonstrated binding ratios of >2.0 (Table 1). These binding ratios indicated WEE virus infection at clinical levels.

TABLE 1. *Radioimmunoassay of WEE virus-infected tissues*

| Specimen[a] | Plaque assay titer[b] (PFU/ml) | I:C ratio[c] |
|---|---|---|
| Optic nerve ..... | $1.5 \times 10^2$ | 3.9 |
| Eye ..... | $5.0 \times 10^2$ | 3.0 |
| Cerebellum ..... | $5.0 \times 10^5$ | 4.2 |
| Choroid plexus .. | $6.5 \times 10^3$ | 4.2 |
| Spinal fluid ..... | $8.0 \times 10^3$ | 3.3 |

[a] Supernatants of tissue homogenates from infected (I) and uninfected (C) monkey fetuses.

[b] Virus titer of supernatants titrated on DEC monolayers; all uninfected tissues were negative for infectious virus. PFU, Plaque-forming units.

[c] Binding ratio as defined in text.

We feel that this procedure can be utilized in clinical laboratories for the rapid and specific identification of WEE virus and can be readily adapted to several other group A arboviruses.

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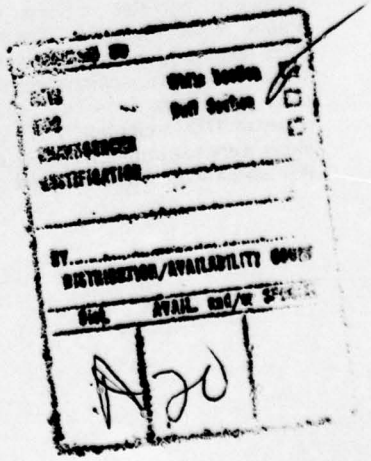
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